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Anaerobic Microbial Activities of a Nigerian Onshore Oil Production Facility that Uses Underground Water with Zero Sulfate Concentration for Injection

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: To evaluate the anaerobic microbial activities and souring potential of a Nigerian onshore oil production facility that uses zero sulfate underground water for injection.
Methodology: Key functional group activities such as the ability to reduce sulfate and generate sulfide by sulfate reducing bacteria (SRB), the ability to reduce nitrate to nitrite by the heterotrophic nitrate reducing bacteria (hNRB) and the ability to reduce nitrate and oxidize sulfide by sulfide oxidizing, nitrate reducing bacteria (so-NRB) were determined in samples using CSB-K medium.
Results: Lactate utilizing SRBs and hNRBs were found to be common in most oil field samples while the activities of so-NRBs were limited to very few samples. It was also observed that the underground water with zero sulfate concentration and negligible microbial activity poses no souring risk to the oil field under investigation. The produced water and oil samples from the facility though with considerable populations and activities of SRBs also recorded negligible concentration of sulfate and some organic nutrients and therefore are not likely to pose some souring risks to the oil facility under investigation.
Conclusion: From our investigation, it is evident that the zero sulfate underground water with negligible SRB populations and activities poses no souring risks to the facility under investigation

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but corrosion risks cannot be completely ruled out since some methanogens that are indigenous to the oil field can initiate corrosion using alternative pathways in the absence of sulfate.

Keywords: SRB; hNRB; so-NRB; souring; produced water; injection water.

1. INTRODUCTION

Multiple group of microorganisms with diverse physiological and metabolic abilities and phylogenetic affiliations have routinely been isolated from oil reservoirs and it is a well-established scientific fact that oil reservoirs harbor and sustain diverse group of bacterial and archaeal communities [1-4]. Despite the numerous researches that have been conducted in the area of oil field microbiology, scientists still need more understanding on the phylogenetic diversity, metabolic capabilities, ecological roles and community dynamics of oil reservoir microbial communities [5,6].

Microorganisms thrive in oil reservoirs under strict anaerobic conditions and the major metabolic processes in oil reservoirs include sulfate reduction, methanogenesis, fermentation, homoacetokinesis and to some extent nitrate reduction especially when nitrate is added to injection water for souring control [7,8]. The anaerobic food chain of oil field microorganisms are therefore based on the use of organic compounds by fermentative bacteria and sulfate reducing bacteria (SRB) oxidizing organic matter under anaerobic conditions and methanogenesis through carbon dioxide reduction and hydrogen scavenging may be the dominant terminal metabolic process [1,9-11]. Potential electron donors for fermentation and sulfate reduction include: acetate, formate, propionate, butyrate and benzoate [1,12,13].

In most subsurface environment as is the case with oil reservoirs, nitrogen and phosphorus are often the main limiting nutrients but nitrogen is unlikely to be limited in petroleum reservoirs since abundant ammonium ions buffered by reservoir minerals should be the primary nitrogen source for in-situ bacterial activity [14]. In addition to the limited availability of nutrients, low redox potential, electron donors and acceptors, temperature and salinity appear to be the most important environmental factors that shape the status of oil reservoir microbial communities [1].

In the present study, detailed anaerobic microbial activities were carried out in an onshore oil production facility in Nigeria that uses low sulfate underground water for injection. Functional group activities such as the ability to reduce sulfate, nitrate, oxidize sulfide and produce hydrogen sulfide in addition to utilization of various organic substrates such as lactate and acetate were used to quantify anaerobic microbiological activities.

2. MATERIALS AND METHODS

2.1 Sample Collection and Shipment

Samples 2N1 (delivery line crude), 2N2 (Crude from HP separator), 2N3 (Injection water), 2N4 (Oily sludge), 2N5 (Treated produced water) and 2N6 (Untreated produced water) were collected from Obigbo North field in sterile 500 ml Nalgene sample bottles which were filled to the brim to exclude air. The samples were later shipped to the Petroleum microbiology research laboratory, University of Calgary for analysis.

2.2 Chemical Analysis

The samples were analyzed for pH, $S0_4^{2-}$,HS^- ,NH_4^+, $N0_3^-$, $N0_2$ and organic acid salts such as acetate, propionate and butyrate.The pH was analyzed using Orion pH meter. SO_4^{2-} was analyzed in two ways, through High Performance Liquid Chromatography (HPLC) and through turbidimetry using $BaCl_2$ [15]. HS^-, a dissolved sulfide was analysed using diamine method [16]. NH_4^+ was analyzed using the indol-phenol method. NO_3^-, NO_2^- and organic acid salts such as acetate, propionate and butyrate were analyzed using HPLC. SO_4^{2-}, NO_3^- and NO_2^- were analyzed using 100 µl of the samples with 400 µl HPLC anion buffer while organic acid analysis used 300 µl of the samples and 20 µl 1 M phosphoric acid.

2.3 Microbiological Assay

The medium that was used for the microbiological assay was Coleville synthetic brine (CSB-K) with composition (g/l) as previously described [17]; NaCl (1.50), $CaCl_2$ x $2H_2O$ (0.21), $MgCl_2$ x $5H_2O$ (0.54), NH_4Cl (0.30), KCl (0.10), KH_2PO_4 (0.05), Resazurin (1%) 2-3 drops.

These chemicals were mixed and dissolved in MQ water in an Erlenmeyer flask and were transferred to a Widdel flask for autoclaving. After autoclaving, more components were added: Trace elements (1 ml), Selenate-tungstate (1 ml), $NaHCO_3$ (1 M) 30 ml, Na_2S (1 M)1 ml, HCl (2 M) 2 ml, pH adjusted to 7.4. The Widdel flask was connected to a gas stream of 90% N and 10% CO_2. About 70 ml of the medium was then aseptically and anaerobically dispensed to 125 ml serum bottles with a gas phase of 90% N and 10% CO_2 and closed with a sterile butyl rubber stopper.

2.4 Components Added to CSB-K for Specific Microbiological Tests

The following electron donors and acceptors were added to the CSB-K medium in serum bottles to determine the functional group activity of major bacterial groups:

a. Sulfate-reducing bacteria (SRB) – 40 mM lactate and 20 mM sulfate; 3 mM volatile fatty acids (VFA) and 20 mM sulphate
b. Heterotrophic nitrate reducing bacteria (hNRB) – 3 mM VFA and 10 mM nitrate
c. Sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) – 5 mM sulfide and 10 mM nitrate

3.5 ml of the samples (5%) were added to the prepared media bottles and incubated at 37°C in a shaker for about 30 days. Using a sterile syringe needle, 1 ml of the sample was taken periodically for every 2 days within the first one week and subsequently for every 7 days and analyzed for sulfide, sulfate, nitrate and nitrite using HPLC. Microbial activities were calculated as $100/t_{1/2}$, where $t_{1/2}$ is the time (days) needed to reduce half of the sulfate (SRB activity), nitrate (hNRB and so-NRB activities) and sulfide concentrations (so-NRB).

2.5 Most Probable Number Test (MPN)

To quantify the presence of SRBs in the samples, API RP-38 broth media were used. Formation of black precipitates of iron sulfide has been used as a diagnostic tool to analyze the presence of SRB. Dilution series of up to 10^{-8} was made to quantify the presence of SRB in the samples. With the use of a syringe needle, 1 ml of the samples was inoculated to the 9 ml medium making a ten-fold dilution. Samples were then incubated at 37°C for up to 30 days.

3. RESULTS

3.1 Chemical Characterization of Samples

Results on chemical analysis of samples showed that all the samples recorded a fairly neutral pH with the exception of sample 2N3 (5.1). No HS^- gas was found in all the samples except sample 2N1 which recorded very negligible concentrations. Sulfate concentration was zero in samples 2N1, 2N2 and 2N3 but relatively low in samples 2N4, 2N5 and 2N6. Nitrite was absent in all the samples analyzed, same goes with nitrate only that samples 2N1, 2N2 and 2N5 recorded very negligible nitrate concentrations. The organic acids measures such as acetate and propionate were more concentrated in samples 2N1 and 2N2 than the rest of the samples. On the contrary, butyrate was found to be more concentrated in sample 2N5 than the rest of the samples. Detailed results are shown in Table 1.

3.2 Microbial Counts using MPN Technique

The MPN results showed the highest SRB concentration of 10^5 in samples 2N1, 2N4 and 2N5 followed by sample 2N6 (10^4). Samples 2N2 and 2N3 recorded relatively low concentration of SRB. Detailed results are shown in Table 2.

Table 1. Chemical analysis of the samples (values in millimolar concentrations)

Sample code	pH	HS^- chemical	SO_4^{2-} chemical	SO_4^{2-} HPLC	NH_4^+ chemical	NO_3^- HPLC	NO_2^- HPLC	Acetate HPLC	Propionate HPLC	Butyrate HPLC
2N1	7.1	0.02	0	0.02	0.17	0.02	0	29.6	2.4	0.4
2N2	7.1	0	0	0.07	0.2	0.05	0	60.0	5.8	1.2
2N3	5.1	0	0	0	0.06	0	0	0.5	1.3	1.8
2N4	7.1	0	2.4	0	0.17	0		0.7	0.6	2.4
2N5	7.1	0	0.5	0	0.16	<0.01	0	2	0.1	5.7
2N6	7.2	0	0.86	0.05	0.18	0	0	1.9	0.3	1.3

3.3 Anaerobic Microbiological Activities of Oil Field Samples with Emphasis on Key Functional Groups

3.3.1 Sample 2N1 (delivery line crude)

Delivery line crude showed high SRB activity showing more utilization of lactate as electron donor than the VFA. SRB with lactate had high activity of 67 units/day compared with 3 units/day for SRB with VFA. On the so-NRB activity, 5 mM sulfide was utilized within 3 days with 1 mM of nitrate being reduced to nitrite. Consumption of nitrate by heterotrophic nitrate-reducing bacteria was very quick as 10 mM nitrate was consumed in ≤ 3 days (Fig. 1).

3.3.2 Sample 2N2 (crude from HP seperator)

Sample 2N2 also showed preference for utilization of lactate than VFA with considerable reduction of sulfate and production of sulfide in lactate media. The so-NRB showed no activity as there was no reduction of nitrate or oxidation of sulfide. hNRB showed some activity of nitrate reduction but no nitrite was observed (Fig. 2)

3.3.3 Sample 2N3 (injection water)

Sample 2N3 which is an underground water with zero sulfate concentration showed no bacterial activity as it relates to utilization of lactate and VFA by SRB, reduction of nitrate by hNRB and so-NRB and oxidation of sulfide by so-NRB (Fig. 3).

3.3.4 Sample 2N4 (sludge tank)

Sludge tank sample (2N4) showed considerable SRB activity with lactate with an activity of 20 units/day. About 75% of the sulfate concentration in lactate was utilized within 7 days. SRB with VFA showed production of sulfide after day 3 and its activity was slow within 31 days of incubation and only 5 mM of sulfate was used up within 31 days. Nitrate was used up within 3 days by hNRB, and further reduction of nitrate was rapid. There was no so-NRB activity (Fig. 4).

3.3.5 Sample 2N5 (produced water after treatment)

Sample 2N5 showed high SRB activity with lactate utilization (50 units/day) and rapid reduction of sulfate and production of sulfide within 3 days. In contrast, SRB in VFA media showed a slower activity of VFA utilization (3 units/day). For so-NRB activity, the entire sulfide was used up within 3 days and total concentration of nitrate was reduced by half after 3 days. hNRB activity showed complete consumption of nitrate within 3 days (Fig. 5).

3.3.6 Sample 2N6 (produced water before treatment)

Sample 2N6 (produced water before treatment) showed a slower activity of SRB when compared with sample 2N5 (produced water after treatment). Like what was observed in previous samples, Lactate media was a better growth substrate for the SRB than the VFA. The activity of hNRB showed nitrate to be completely consumed within 3 days which resulted in its reduction to nitrite. There was no considerable activity of so-NRB (Fig. 6)

Table 2. Most probable number results of samples

Sample code	Sample description	# of SRB per ml
2N1	Delivery line crude	10^5
2N2	Crude from HP separator	10
2N3	Injection water	10
2N4	Sludge tank 1201B	10^5
2N5	Produced water sample after treatment	10^5
2N6	Produced water sample before treatment	10^4

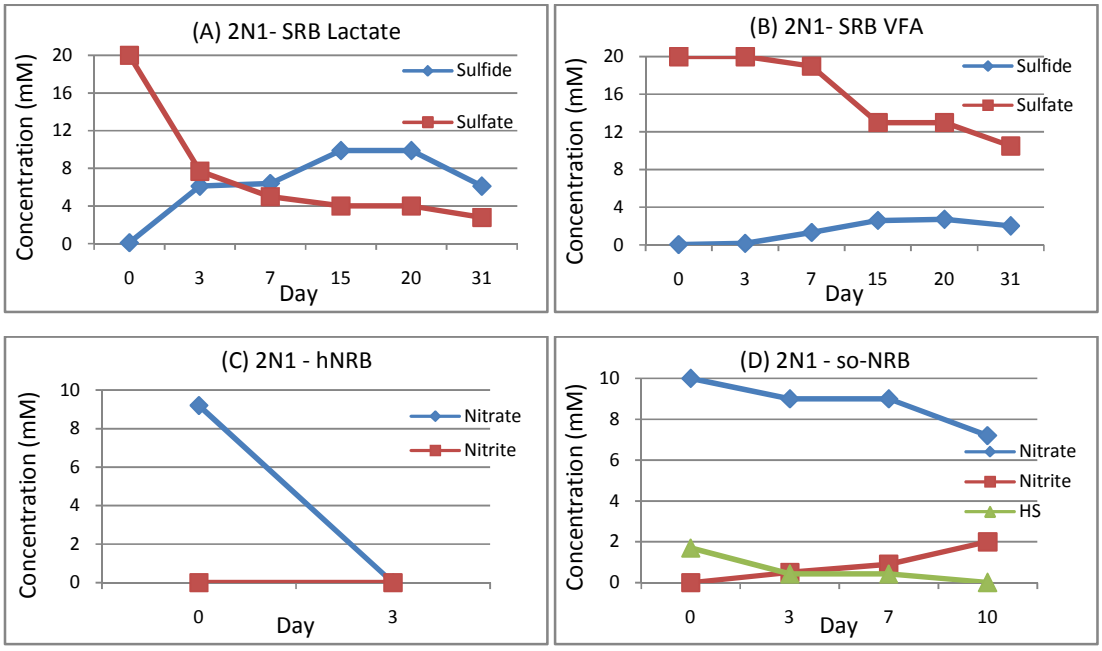

Fig. 1. 2N1 (Delivery line crude) microbial activities: (A) SRB activities showing sulfide and sulfate concentrations in lactate substrate; (B) SRB activities showing sulfide and sulfate concentrations in VFA substrate; (C) bacterial activity of heterotrophic, nitrate-reducing bacteria (hNRB) showing nitrate and nitrite concentrations; (D) sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) showing sulfide, nitrate and nitrite concentrations

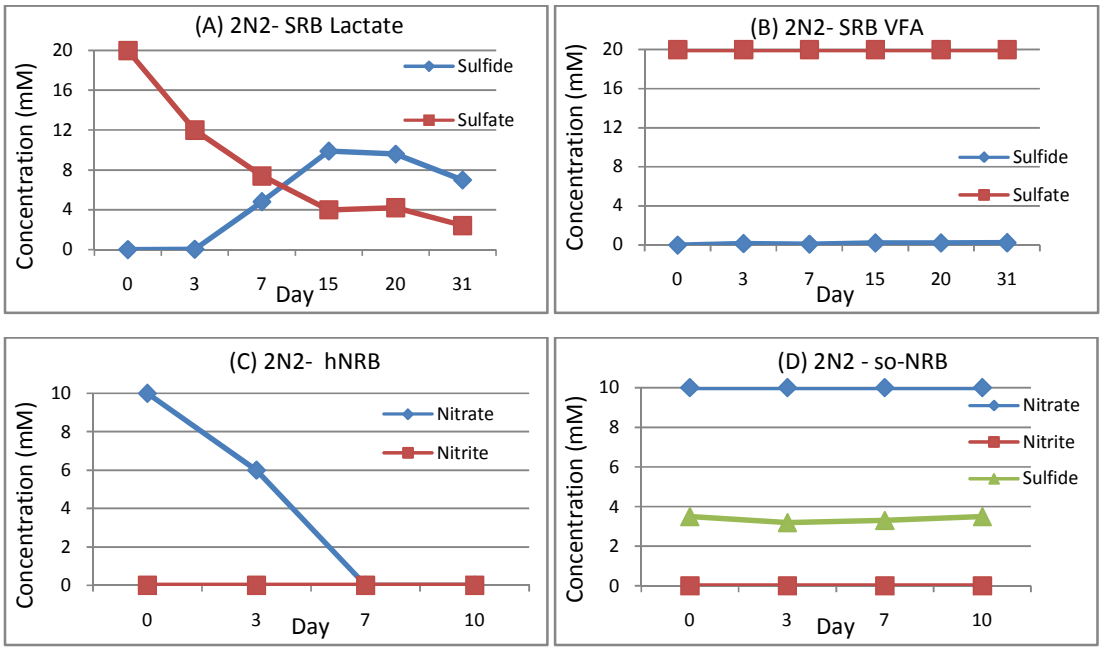

Fig. 2. 2N2 (crude from HP separator) microbial activities: (A) SRB activities showing sulfide and sulfate concentrations in lactate substrate; (B) SRB activities showing sulfide and sulfate concentrations in VFA substrate; (C) bacterial activity of heterotrophic, nitrate-reducing bacteria (hNRB) showing nitrate and nitrite concentrations; (D) sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) showing sulfide, nitrate and nitrite concentrations

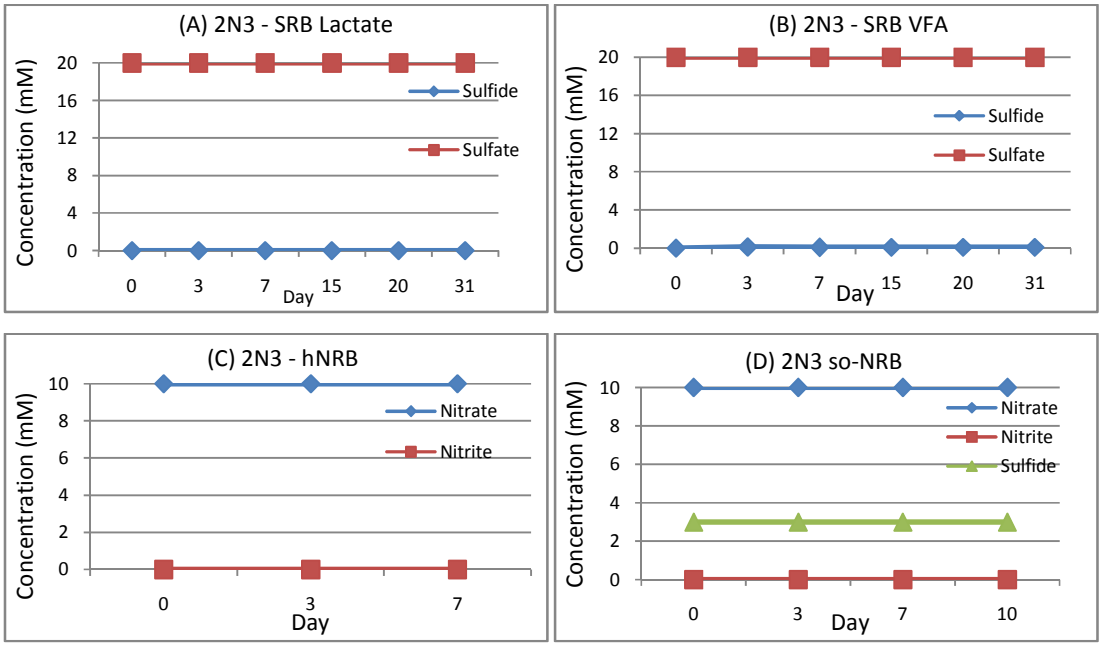

Fig. 3. 2N3 (injection water) microbial activities: (A) SRB activities showing sulfide and sulfate concentrations in lactate substrate; (B) SRB activities showing sulfide and sulfate concentrations in VFA substrate ;(C) bacterial activity of heterotrophic, nitrate-reducing bacteria (hNRB) showing nitrate and nitrite concentrations; (D) sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) showing sulfide, nitrate and nitrite concentrations

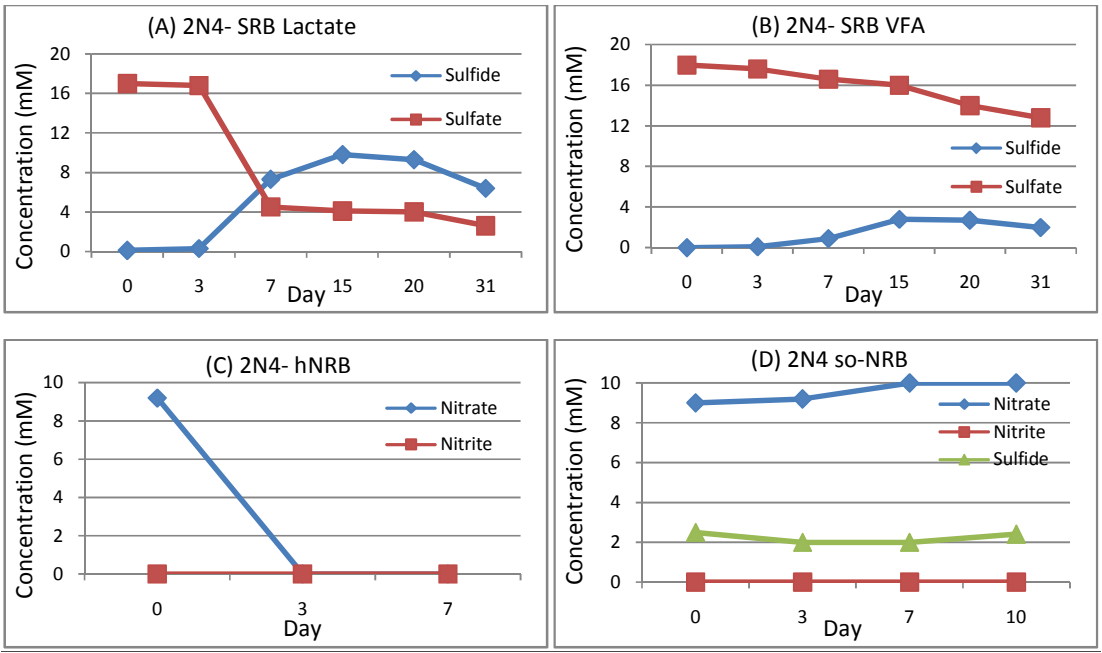

Fig. 4. 2N4 (sludge tank) microbial activities: (A) SRB activities showing sulfide and sulfate concentrations in lactate substrate; (B) SRB activities showing sulfide and sulfate concentrations in VFA substrat; (C) bacterial activity of heterotrophic, nitrate-reducing bacteria (hNRB) showing nitrate and nitrite concentrations; (D)sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) showing sulfide, nitrate and nitrite concentrations

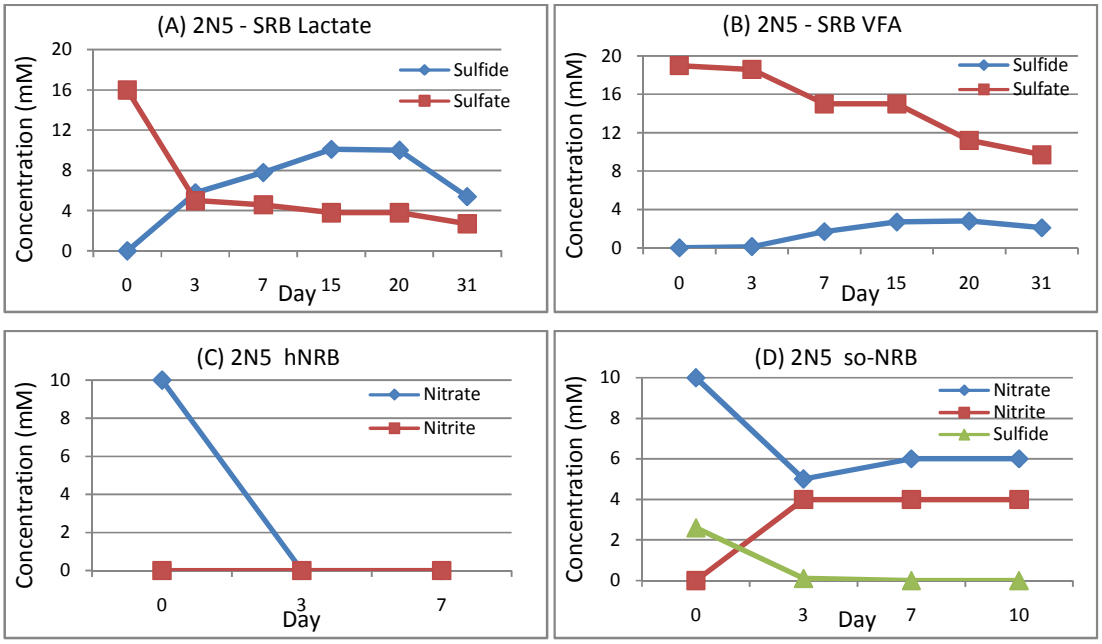

Fig. 5. 2N5 (produced water sample after treatment) microbial activities: (A) SRB activities showing sulfide and sulfate concentrations in lactate substrate; (B) SRB activities showing sulfide and sulfate concentrations in VFA substrate; (C) bacterial activity of heterotrophic, nitrate-reducing bacteria (hNRB) showing nitrate and nitrite concentrations; (D) sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) showing sulfide, nitrate and nitrite concentrations

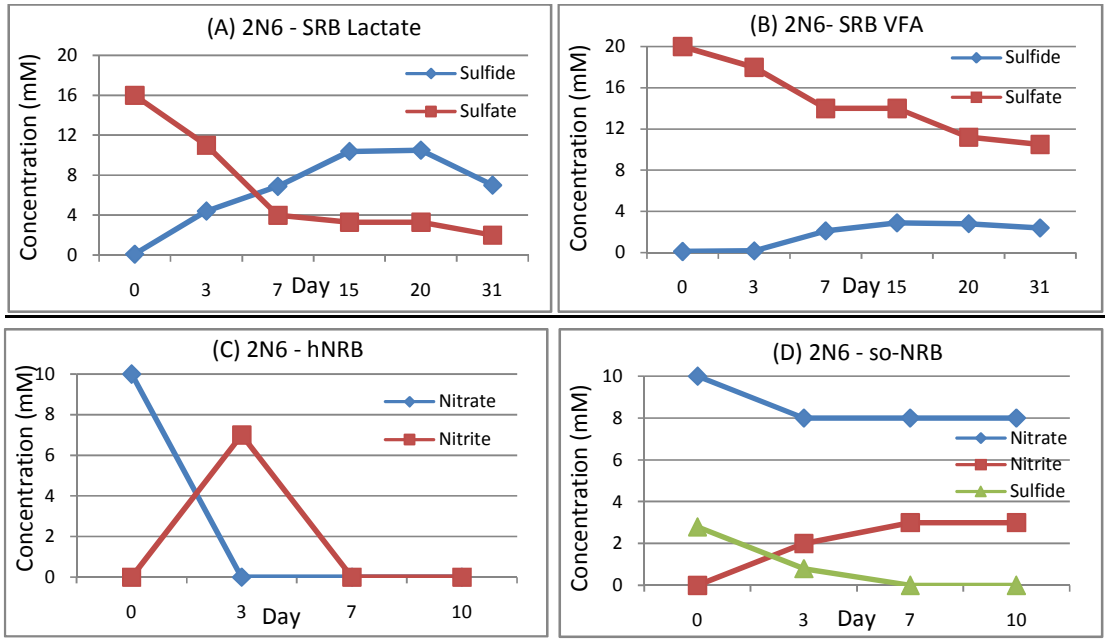

Fig. 6. 2N6 (produced water sample before treatment) microbial activities: (A) SRB activities showing sulfide and sulfate concentrations in lactate substrate; (B) SRB activities showing sulfide and sulfate concentrations in VFA substrate; (C) bacterial activity of heterotrophic, nitrate-reducing bacteria (hNRB) showing nitrate and nitrite concentrations; (D) sulfide-oxidizing, nitrate-reducing bacteria (so-NRB) showing sulfide, nitrate and nitrite concentrations

4. DISCUSSION

Physico-chemical analysis of samples revealed that the onshore crude samples (2N1 and 2N2) recorded negligible concentrations of sulfate, ammonium ions and nitrate but considerable concentrations of VFA (acetate, propionate and butyrate). The underground water used for injection which was slightly acidic (pH=5.1), recorded zero concentration of sulfate and nitrite and negligible concentrations of ammonium ions, nitrate and VFA. The produced waters (2N5 and 2N6) also recorded low concentrations of sulfate, ammonium ions, nitrate and VFA. The negligible concentrations of sulfate and organic nutrients (VFA) observed in injection and produced water samples poses a much lower souring risk in the oil field when compared with offshore oil operations that uses sea water rich in sulfate for injection. Our observation was similar to that of [6] where which stated that most aquifer waters used for injection in most onshore oilfield operations contain much lower sulfate concentration than sea water with low concentration of organic nutrients and these factors confers a very low souring risk to such operations. We also observed that despite the low concentrations of sulfate and organic nutrients in the samples, SRBs were present at considerable populations in the produced waters and oil samples but relatively low in the injection water.

On the anaerobic microbiological activities of oil field samples with emphasis on functional group activities, we observed relatively high SRB activity with subsequent sulfate reduction and production of hydrogen sulfide in in samples 2N1 (Delivery line crude), 2N2 (Crude from HP separator), 2N4 (Sludge tank), 2N5 (Treated produced water) and 2N6 (Untreated produced water). It was also observed that lactate was a more preferred growth media in all the samples than VFA. hNRB also showed considerable bacterial activities in samples 2N1, 2N2, 2N4, 2N5 and 2N6. According to [17], lactate utilizing SRBs and hNRBs are common in oil fields. [18, 19] have also advanced that SRBs and hNRBs are widely distributed in oil fields while the distribution of so-NRBs are limited. These assertions are in agreement with our observations because while most of the samples showed considerable SRB and hNRB activities, the activities of so-NRB were limited to few samples (2N1 and 2N5). However, there have been some reported cases of so-NRB isolation from oil fields [20]. The underground injection water sample (2N3) that recorded zero sulfate concentration also showed little or no anaerobic microbial activity as it relates to utilization of lactate and VFA by SRB, reduction of nitrate by hNRB and so-NRB and oxidation of sulfide by so-NRB.

5. CONCLUSION

We have been able to demonstrate from our investigation that the zero sulfate underground water with negligible SRB populations and activities poses no souring risks to the oil field under investigation but corrosion risks cannot be completely ruled out since some methanogens that are indigenous to the oil field are present and they have potential to initiate corrosion using alternative pathways in the absence of sulfate.

We also conclude that the indigenous SRBs that are present at considerable populations in crude oil and produced water samples from the oil fields must have derived their energy for metabolism from the residual VFAs present in the samples but like the injection waters, there was no souring risk because of negligible concentration of sulfate in-situ.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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